भारतीय मानक
Indian Standard

IS 16527 : 2018
(Reaffirmed 2022)

स्टेवियोल ग्लाइकोसाइड, खाद्य ग्रेड — विशिष्टि

Steviol Glycosides, Food Grade — Specification

ICS 67.220.20

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March 2018 **Price Group 2**

Food Additives Sectional Committee, FAD 8

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council.

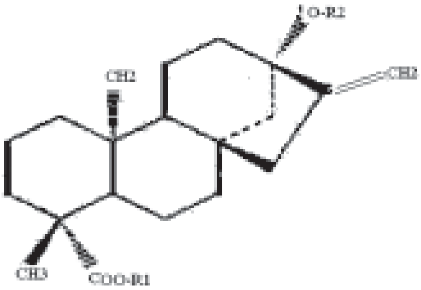
With the increased production of processed foods, manufacturers have started adding a large number of substances generally in small quantities, to improve the appearance, flavour, texture or storage properties of the processed foods. As impurities in these substances have been found to be harmful, it is necessary to have a strict quality control of these food additives. Steviol glycosides are obtained from the leaves of Stevia rebaudiana Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Steviol glycoside extracts are mainly used as a sweetener in the manufacture of fruit and milk drinks, desserts, yoghurt, cold confectionary, delicacies and pickles. Stevioside and Rebaudioside A are reasonably stable under the elevated temperatures used in food processing and do not undergo browning or caramelization when heated.

Steviol Glycosides are about 200 - 300 times sweeter than sucrose.
Synonyms — INS no.960
CAS Number - Stevioside: 57817-89-7 Rebaudioside A: 58543-16-1
Functional uses— Sweetner

Chemical Name —	Stevioside:	13-[(2-0-β -D-glucopyranosyl- β -D glucopyranosyl)oxy] kaur-16-en-18-oic acid, β -D-glucopyranosyl ester
	Rebaudioside A:	13-[(2-0- β -D-glucopyranosyl-3-0- β -D-glucopyranosyl- β -D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β - D-glucopyranosyl ester

Empirical Formula — Stevioside: $C_{38}H_{60}0_{18}$ Rebaudioside A: $C_{44}H_{70}0_{23}$
Molecular Weight — Stevioside: 804.88 Rebaudioside A: 967.03
Structural Formula:



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Indian Standard

STEVIOL GLYCOSIDES, FOOD GRADE — SPECIFICATION

1 SCOPE

This standard prescribes the requirements and the methods of sampling and test for steviol glycosides, food grade.

2 REFERENCES

The following standards contain provisions, which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No	*Title*
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1994	Methods of sampling and test for food colours (*second revision*)
2491 : 1998	Code for hygienic conditions for food processing units (*second revision*)
7928 : 1992	Alginic acid, food grade — Specification

3 REQUIREMENTS

3.1 The material shall be a white to light yellow powder, odourless or having a slight characteristic odour.

3.2 The material shall conform to the requirements prescribed in Table 1.

4 PACKING, STORAGE AND MARKING

4.1 Packing

The material shall be filled in containers with as little air space as possible. The containers shall be such as to preclude air contamination of the contents with metals or other impurities.

4.2 Storage

The material shall be stored in a cool and dry place so as to avoid excessive exposure to heat.

4.3 Marking

Each container shall be marked legibly to give the following information:

a) Name of the material including the words 'Food Grade';

b) Source of manufacture;

c) Minimum net contents;

d) Batch or code number;

e) Date of manufacture; and

Table 1 Requirement for Steviol Glycosides

(*Clause* 3.2)

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Annex of this Standard	Clause of relevant IS
(1)	(2)	(3)	(4)	(5)
1	Total of nine steviol glycosides, percent by mass, *Min*	95.0	A	—
2	*p*H	4.5-7.0	B	—
3	Total ash, percent by mass, *Max*	1.0	C	—
4	Loss on drying at 105°C at 2 h, percent by mass, *Max*	6.0	—	**A-2** of IS 7928 : 1992
5	Residual solvents: a) methanol, mg/kg, *Max* b) ethanol, mg/kg, *Max*	200 5 000	D	—
6	Arsenic, mg/kg, *Max*	1.0	—	**15** of IS 1699 : 1995
7	Lead, mg/kg, *Max*	1.0	—	**15** of IS 1699 : 1995

f) Any other details required under the under the *Legal Metrology (Packaged commodities) Rules*, 2011 and *Food Safety and Standards (Packaging and Labelling) Regulation*, 2011.

4.4 BIS Certification Marking

The product may also be marked with the Standards Mark.

4.4.1 The use of the standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

5 SAMPLING

The representative samples of the material shall be drawn and conformity of the material to the requirements of this specification shall be determined according to the procedure prescribed in **4** of IS 1699.

6 TESTS

6.1 Tests shall be carried out by the methods specified in ***3.2*** and cols 4, 5 and 6 of Table 1.

6.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the result

ANNEX A

[*Table* 1, *Sl No.* (i)]

DETERMINATION OF STEVIOL GLYCOSIDES

A-0 Determine the percentages of the individual steviol glycosides by HPLC under the following conditions.

A-1 REAGENTS

A-1.1 *Acetonitrile*, more than 95 percent transmittance at 210 nm.

A-1.2 *Standards. Stevioside*, more than 99.0 percent purity on the dried basis. Rebaudioside A: more than 99.0 percent purity on the dried basis. Mixture of nine steviol glycosides standard solution: Containing stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside. This solution is diluted with water-acetonitrile (7:3) accordingly and is used for the confirmation of retention times.

A-1.3 *Standard Solution*, Accurately weigh 50 mg of stevioside and rebaudioside A standard into each of two 50-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

A-1.4 *Sample Solution*, Accurately weigh 50-100 mg of sample into a 50-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

A-2 PROCEDURE

Inject 5 µl of sample solution under the following conditions. Column: Capcell pak C18 MG II or Luna 5 C18 (2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter: 4.6 mm, particle size: 5m)

A-2.1 Conditions

Mobile phase : 32:68 mixture of acetonitrile and 10 mmol/lL sodium Phosphate buffer (pH 2.6)

Flow rate : 1.0 ml/min

Detector : UV at 210 nm

Column temperature: 40° C

Record the chromatogram for about 30 min.

A- 2.2 Identification of the Peaks

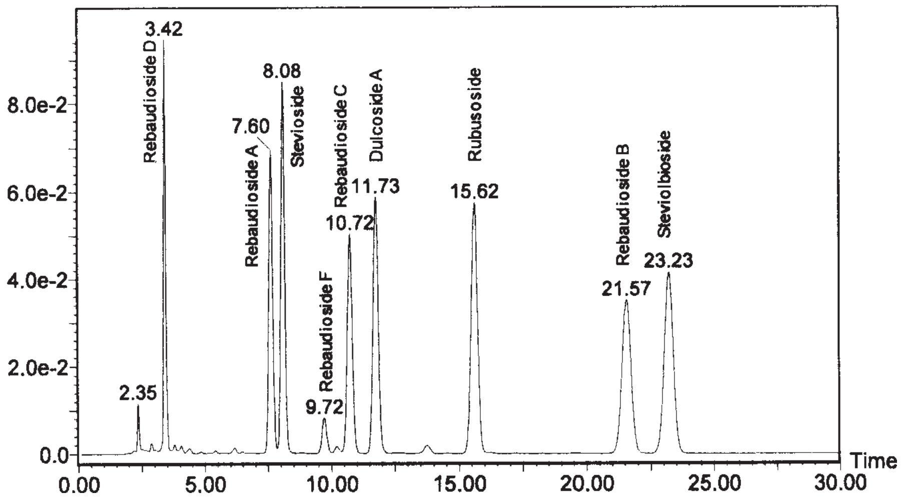
Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of nine steviol glycosides standard solution (*see* Fig. 1). Measure the peak areas for the nine steviol glycosides from the sample solution.

Measure the peak area for stevioside and rebaudioside A from their standard solutions.

A-3 CALCULATION

Calculate the percentage of each of the eight steviol glycosides except rebaudioside A in the sample from the formula:

$$\text{Percent } X = \frac{[Ws]}{W} \times \frac{[f_x \times A_x]}{As} \times 100$$



Concentration : 0.5 mg/ml each except reabudioside F (about 0.1 mg/ml)

FIG. 1 CHROMATOGRAM OF MIXTURE OF NINE STEVIOL GLYCOSIDES STANDARD SOLUTION
COLUMN : CAPCELL PAK C18 MG II

Calculate the percentage of rebaudioside A in the sample from the formula:

$$\text{Percent Rebaudioside A} = \frac{[W_R]}{W} \times \frac{[A_x]}{A_R} \times 100$$

where

X = steviol glycoside;

W_S = amount (mg) calculated on the dried basis of stevioside in the standard solution;

W_R = amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;

W = amount (mg) calculated on the dried basis of sample in the sample solution;

A_S = peak area for stevioside from the standard solution;

A_R = peak area for rebaudioside from the standard solution;

A_x = peak area of X for the sample solution; and

f_x = ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides as sum the nine percentages.

ANNEX B

[*Table* 1, *Sl. No* (ii)]

DETERMINATION OF *p*H

B-1 APPARATUS

B-1.1 *p*H Meter

Use a standard laboratory pH meter (*see* IS 2711).

B-1.2 PROCEDURE

Dissolve 1 g of the sample in 100 ml water. Measure the *p*H of the solution using a standard *p*H meter. Repeat the test and record the mean *p*H value.

ANNEX C

[*Table* 1, *Sl. No* (ii)]

DETERMINATION OF ASH

C-1 PROCEDURE

Weigh accurately about 3 g of the material previously dried at 105°C for 4 h, in a tared crucible and incinerate at a low temperature, not exceeding a dull red heat until free from carbon. Cool the crucible and its content in a desiccator, weigh, and determine the weight of ash.

ANNEX D

[*Table* 1, *Sl No.* (iii)]

DETERMINATION OF RESIDUAL SOLVENTS USING HEAD SPACE CHROMATOGRAPHY (DETERMINATION CARRIED OUT IN WATER)

D-1 REAGENTS

D-1.1 Blank, sample with very low solvent content

D-1.2 Internal standard, 3-methyl-2-pentanone

D-1.3 Methanol

D-1.4 Demineralised Water

D-1.5 Internal Standard Solution, Add 50.0 ml water to a 50 ml injection vial and seal. Accurately weigh and inject 15 (µl 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.

D-1.6 Blank solution, Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of water and 1.0 ml of the internal standard solution. Heat at 60°C for 10 min and shake vigorously for 10 sec.

D-1.7 Samples, Weigh accurately 0.20 g sample into an injection vial. Add 5.0 ml water and add 1.0 ml of the internal standard solution. Heat at 60°C for 10 min and shake vigorously for 10 s.

D-1.8 Calibration Solution — Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of the water and 1.0 ml of the internal standard solution. Weigh the vial accurately to within 0.01 mg. Inject a known volume of the component of interest through the septum and again reweigh the vial. Heat at 60°C for 10 min and shake vigorously for 10 s.

D-2 PROCEDURE

Place the sample, blank and calibration samples in the sample tray of the head-space gas chromatograph - FID system. Analyse using the following analytical conditions.

D-2.1 Column

Fused silica, length 0.8 m, i.d. 0.53mm, coated with DB-wax, film thickness 1 µm.

D-2.2 Coupled with, Fused silica, length 30 m, i.d. 0.53 mm, coated with DB-1, film thickness 5 µm.

D-2.3 Conditions

Carrier gas: Helium
Flow rate: 208 kPa, 5 ml/min
Detector: FID
Temperatures
  Injector: 140°C
  Oven conditions: 35°C for 5 min, then 5°C/min to 90°C, then 6 min at 90°
  Detector: 300°C
Head space sampler
Sample heating temperature: 60°C
Sample heating period: 10 min
Syringe temperature: 70°C
Transfer temperature: 80°C
Sample gas injection: 1.0 ml in split mode

D-3 CALCULATION

$A \times B \times C / 50$ = mg component per injection vial

where

A = relative peak area of the component concerned;
B = internal standard, in mg; and
C = calibration factor;
Calibration factor = $D \times 50 / (E \times (F - G))$

where:

D = component weighed, in mg;

E = internal standard, in mg;

F = relative peak area of component for the calibration solution; and

G = relative peak area of the same component for the blank solution.

(*Continued from second cover*)

The nine Steviol Glycosides are as under:

Compound name	R1	R2
Stevioside	β-Glc	β -Glc β -Glc (2→ 1)
Rebaudioside A	-Glc	β -Glc- β -Glc(2→ 1) I -Glc(3→ 1)
Rebaudioside B	H	-Glc- β -Glc(2→ 1) I -Glc(3→ 1)
Rebaudioside C	-Glc	β -Glc-a-Rha(2→ 1) I –Glc (3
Rebaudioside 0	β -Glc- β –Glc (2→ 1)	β -Glc- β -Glc(2→ 1) I β -Glc(3→ 1)
Rebaudioside F	β -Glc	β -Glc- β –Xyl (2→ 1) I β -Glc(3→ 1)
Dulcoside A	β -Glc	β -Glc-α-Rha(2→ 1)
Rubusoside	-Glc	β -Glc
Steviolbioside	H	β -Glc- β -Glc(2→ 1)

Steviol (R 1 = R2 = H) is the aglycone of the steviol glycosides. Glc, Rha and Xyl represent, respectively, glucose, rhamnose and xylose sugar moieties.

In the formulation of this standard, due consideration has been given to the *Legal Metrology (Packaged Commodities) Rules*, 2011 and *Food Safety and Standards Act*, 2006 and regulations framed there under. The standard is however subject to restrictions imposed under these rules, wherever applicable.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

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Amendments Issued Since Publication

Amendment No.	Date of Issue	Text Affected

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